MEIOSIS AND CHIASMA FORMATION IN PÆONIA SUFFRUTICOSA

KARL SAX

With plate 52 and two text figures

THE GENETIC evidence has shown conclusively that crossing over occurs at the four strand stage and between only two of the chromatids at any one locus (Bridges and Anderson 1925, et al.). An actual physical interchange of chromosome segments has been shown to be correlated with genetic crossing over in *Drosophila* (Stern, 1931) and in *Zea* (Creighton and McClintock, 1931). Such an interchange of chromosome segments should invariably produce an asymmetrical arrangement of the chromatids. If chiasmata are produced by previous crossovers, each chromatid will not always lie in the same quadrant at all loci. A study of chromatid organization at meiosis should throw some light on the nature of chiasma formation.

In this discussion the term *chiasma* is used to denote an apparent change of partners among the chromatids at meiosis without reference to the origin of such configurations.

If chiasmata are caused by the alternate pairing of sister and non-sister chromatids, the sister chromatids will be paired on one side of a chiasma, and homologous threads will be associated on the other side of the chiasma, as shown in diagram 1 (Fig. 1). Viewed from one angle, two of the chromatids seem to cross each other, but from another angle the other two chromatids form the cross. The apparent cross at each chiasma is formed by diagonal rather than adjacent chromatids. Each chromatid will lie in the same quadrant at all loci if no crossovers or twists in the chromatids have occurred. The pairs of chromatids on either side of a chiasma would be expected to open out at right angles to each other.

Chromosome configurations which seem to support this interpretation of chiasma formation have been described and pictured by Wenrich (1916), Robertson (1916), McClung (1927), Wilson (1925), Bělař (1928), Carothers (1927), Taylor (1930), and others. Only in a relatively few cases is there any twisting of paired chromatids in the meiotic chromosomes pictured by these investigators, and in most cases each chromatid lies in the same quadrant at all loci. As McClung has pointed out, the clearest figures shown by Jannsens (1924) also show the chromatids in the same quadrants at all loci.

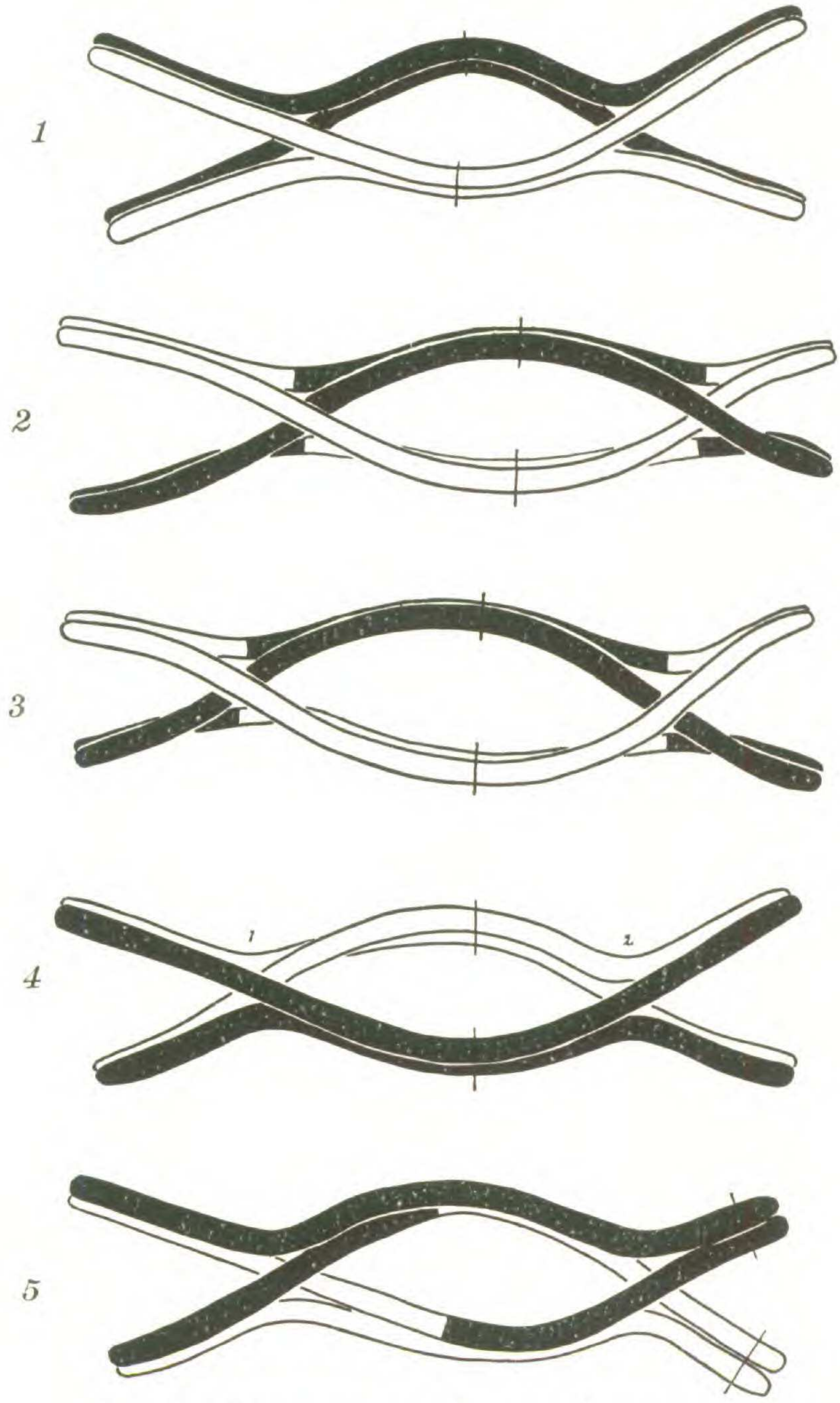

FIG. 1. DIAGRAMS OF CHIASMA FORMATION.

One pair of sister chromatids is represented by white rods and the other pair by black rods. The spindle fiber attachment points are indicated by cross lines. The term *chiasma* is used to designate the change of partners among the four chromatids of a meiotic chromosome without reference to its origin.

Diagram 1. Chiasma formation as interpreted by McClung and others. A chiasma is caused by the alternate opening out of sister and non-sister chromatids in pairs. As viewed from the end, each chromatid maintains its position in the same quadrant at all loci. Alternate internodes lie in planes at right angles to each other.

Diagrams 2 and 3. Chiasma formation according to Jannsens' partial chiasmatypy hypothesis. Each chiasma is the result of a previous crossover between two of the four chromatids. Two of the chromatids do not maintain their position in the same quadrants at all loci. The pairs of chromatids on both sides of a chiasma should tend to lie in the same plane.

Diagram 4. Chiasma formation according to McClung's interpretation, but with 2 chromatids in different quadrants at different loci, due to a half-twist in one pair of chromatids before pairing.

Diagram 5. A chiasma similar to number 1 of diagram 4, but the half twist is caused by a crossover resulting from breaks in two chromatids of a previous median chiasma. A bivalent of this type might lead to interlocking of homologous chromosomes at the first meiotic metaphase, and in this respect resembles the configuration shown in diagram 2.

Darlington (1930, figs. 8, 9, 10) and Moffett (1932, diagrams 2A, 2B), have published diagrams of chiasma formation which are in accord with McClung's interpretation. More recently Darlington and Dark (1932) have shown figures of bivalent chromosomes of *Stenobothrus* where the relations of the four chromatids can be observed at all loci in some of the chromosomes. The chromatids in the shorter chromosomes usually show a symmetrical arrangement, as would be expected if chiasmata are produced by alternate opening out of sister and homologous chromatids at diplotene. In the long bivalents pictured by Darlington and Dark it is difficult to follow the position of the chromatids at all loci, but some of the chiasmata in these chromosomes are clearly symmetrical, although some bivalents (D, fig. 6) do show asymmetrical arrangements of the chromatids. The double chiasmata analyzed were always (?) "compensating" (reciprocal) and never diagonal (equational). In *Drosophila* both types of crossovers occur with about equal frequency.

According to Jannsen's (1924) partial chiasmatypy hypothesis, a chiasma is produced by crossing over between two of the four chromatids (Fig. 1, diagrams 2 and 3). This interpretation has recently been sponsored by Belling (1931), Darlington (1931), and others. In all cases it is assumed that sister chromatids are paired at all loci, and that each chiasma represents a crossover. As shown in diagrams 2 and 3, two chromatids would not lie in the same quadrant at all loci, and the two chromatids which form the cross should be adjacent and not diagonal. At diplotene and subsequent stages the pairs of chromatids should lie in the same plane on either side of a chiasma and not at right angles to each other. As shown in another paper (Sax 1932), it is also necessary to assume, on Belling's hypothesis, that crossovers occur between sister chromatids which would produce many twists in paired sister chromatids between chiasmata. Certain types of double crossovers should result in interlocking of homologous chromosomes at metaphase, as would be the case if the spindle fiber were terminal in diagram 2 (Fig. 1). It is possible, of course, that the movement of the chromatids would occasionally result in a symmetrical configuration where each chromatid would lie in the same quadrant at all loci, but such figures should be rare.

The most critical studies of chromatid organization and the nature of chiasma formation have been based on preparations of Orthopteran chromosomes. In this group of animals the four chromatids can often be recognized at all loci at the diplotene stage. In plant species, however, the chromatids are usually closely paired in spiral chromonemata during late prophase and early metaphase stages so that the individual chromatids can seldom be differentiated until the anaphase stage of meiosis. Newton's (1926) figures of *Tulipa* and *Fritillaria* chromosomes do show the tetrad nature of the meiotic chromosomes to some extent. According to Newton, the hypothesis "which explains the diakinetic figures as due to the opening out in two planes at right angles of what are originally four parallel chromatids, is adequate to explain the events of diakinesis and division in *Tulipa* and *Fritillaria*." Taylor's (1930) figures of *Gasteria* chromosomes show clearly the alternate opening out of the chromatids in pairs at right angles to each other, with each chromatid in the same quadrant at all loci. Some of his figures also show the chromatids opening out in pairs at the proximal end, but at the distal end all four chromatids are paired. Such chromosome pairing without chiasma formation can not be reconciled with Darlington's (1931) theory of chromosome pairing at meiosis. According to Darlington, chromosomes are associated only by chiasmata at the first meiotic division. In many species bivalent chromosomes are apparently associated only by chiasmata at the meiotic metaphase.

In numerous species of plants it is clear that alternate internodes of meiotic chromosomes are oriented in planes at right angles to each other, as is the case in the multiple ring chromosomes of Orthopteran species.

CHIASMA FORMATION IN PÆONIA

Pæonia suffruticosa has five pairs of large chromosomes. The haploid set of chromosomes at metaphase of the microspore division is shown in text-figure 2. Four of the chromosomes have an approximately median spindle fiber constriction, while the fifth chromosome has a subterminal fiber attachment with a trabant at the end of the short arm. The somatic chromosomes are long and slender, and even at metaphase the paired chromatids are often twisted about each other (Figure 1 of pl. 52). If the sister chromatids of homologous chromosomes are twisted at the time of pairing at meiosis, then the chromatids will have an asymmetrical arrangement at the diplotene stage. Such meiotic chromosomes should often show half twists in paired chromatids, even if no crossovers have occurred.

The chromosomes at the meiotic prophase were so diffuse and granular in appearance that any critical study of early chiasma formation was impossible. Relatively few chiasmata seem to be present, even at the early prophase. At diakinesis the number of nodes or chiasmata can be clearly observed, but the relation of the chromatids was obscure.

FIG. 2. CHROMOSOMES IN PÆONIA MICROSPORE.

The four chromatids can usually be observed at the first meiotic metaphase, and in many cases the relations of the chromatids can be determined at all loci. The chromatids are always closely associated in pairs at the spindle fiber attachment point, but at other loci they may be well separated.

Many of the meiotic chromosomes are paired without the formation of chiasmata (Figures 4a, 4d, 7a, 10b of pl. 52). The sister chromatids appear to be paired throughout their length with no exchange of partners at any locus. At the proximal ends the chromatids have opened out in pairs, but at the distal ends all four chromatids seem to be paired. If non-sister chromatids in such figures were to open out in pairs at the distal ends, then we would expect a chiasma to be formed as shown in figure 9 (pl. 52). Only in rare cases do the distal ends of the chromatids open out to form the cross-shaped figure which is so typical in many other genera. In many cases, however, there is some separation of the paired chromatids at the distal ends of the chromosomes (Figures 2, 5, 11a of pl. 52). Homologous chromosomes which are paired at both ends usually show the sister chromatids paired only at the spindle fiber attachment and pairing of non-sister threads only at the distal ends (Figures 4b, 6b, 7c, 7d of pl. 52).

Half twists in paired chromatids are often observed (Figures 2a, 3d, 4a, 8a, 10a of pl. 52). Such half twists between the fiber attachment and the first chiasma would be expected even if no crossing over occurs.

If crossovers have occurred in the meiotic chromosomes, there should be half twists or an asymmetrical arrangement of the chromatids, but in many cases the chromatids are symmetrical and lie in the same quadrants at all loci (Figures 4c, 4d, 5, 6b, 7a, 7b, 7d of pl. 52). In the chromosomes with a single chiasma it is possible that any earlier asymmetrical relations of the chromatids might be straightened out, in some cases, by the movements of the chromatids during the early stages of division. In chromosomes with a median fiber attachment and two terminal chiasmata, such a change in the relation of the chromatids would be improbable. In some of these chromosomes there are half twists in the chromatids (Figures 4b, 7c, and 8a of pl. 52), but other bivalents show the symmetrically arranged chromatids in the same quadrants at all loci (Figures 6b and 7d of pl. 52). Half twists in the chromatids of such chromosomes may be due either to twisting before the homologues pair or to crossing over. No crossovers could have occurred in the symmetrical chromosomes, even though two chiasmata are present in each pair of chromosomes. Although these chromosomes do not have the usual types of chiasmata, the situation is essentially the same as in the multiple ring chromosomes in Orthoptera where each chromatid lies in the same quadrant at all loci (Fig. 1, diagram 1). Such figures are very difficult to interpret on the basis of the partial chiasmatypy hypothesis.

There are, however, some chromosome configurations which do support the partial chiasmatypy hypothesis. In these chromosomes the chiasma cross is formed by chromatids which were adjacent, rather than diagonal, at the four strand stage (Figures 6a and 11b of pl. 52). In these figures the two upper chromatids form the cross and lie above the other two chromatids on each side of the cross. Such a relation of the chromatids should be characteristic if chiasmata are caused by previous crossovers, as shown in diagrams 2 and 3 (Fig. 1).

This type of chiasma is difficult to account for on the hypothesis that chiasma formation precedes crossing over. If no twists occur in pairs of sister chromatids, the chromatids which form the cross will be diagonal on each side of the chiasma (Fig. 1, diagram 1). If a half twist occurs in one pair of sister chromatids, the chromatids which form the cross will be adjacent on one side of the chiasma, but will be diagonal at the distal end, as shown in diagram 4 (Fig. 1). A rotation of the chromatids in the lower left-hand arm of this chromosome would produce a configuration like those found in figures 6a and 11b (Pl. 52), but such behavior of the chromatids might be expected only in rare cases.

The homologous chromosomes occasionally fail to pair or are separated before the first meiotic metaphase. The univalent chromosomes may pass to opposite poles (Figure 4 of pl. 52), or they may pass to the same pole (Figures 8 and 12 of pl. 52). The segregation of both homologous univalents to the same pole will result in genetic "non-disjunction" if the gametes are viable. (For further discussion, see previous paper, Sax 1932.)

Occasionally a bivalent chromosome divides with apparent difficulty, even when there is no interlocking of paired chromatids. In figure 12 (Pl. 52) a bivalent is shown held together by the attachment of two single chromatids. Such figures are common in many species.

No interlocking of homologous chromosomes was observed in *Pæonia*, and none would be expected where only one chiasma is formed between the spindle fiber and the distal end of the chromosome. Where several chiasmata are formed, some interlocking of chromosomes would be expected, and such types of bivalents are found in the Orthoptera, and in *Lathyrus* and *Lilium*. If chiasmata are formed by previous crossovers, the configuration shown in diagram 2 should result in interlocked chromosomes if the spindle fiber attachment were terminal. On the writer's hypothesis a crossover between two unbroken chiasmata should also result in interlocked homologues at metaphase (Fig. 1, diagram 5).

PRE- AND POST-REDUCTION

The genetic evidence shows that in *Drosophila* the sister chromatids are always paired at the spindle fiber attachment point during the first meiotic division (Bridges and Anderson 1925, et al.). In numerous species of plants and animals the paired chromatids are associated only at the point of spindle fiber attachment at the anaphase and telophase stages of the first meiotic division, and this association is often especially clear during interphase. Since the spindle fiber attachment points can sometimes be observed soon after the chromosomes pair and before the diplotene stage, it seems probable that sister chromatids are usually held together at the fiber constriction, as is clearly the case in *Drosophila*. Such an association of chromatids would mean that the first meiotic division is reductional at the fiber constriction and for all loci between the fiber constriction and the first crossover. Between the first crossover and the distal end of the bivalent chromosome the division is equational. If a second crossover occurs on the same side of the spindle fiber, the loci distal to the second crossover would undergo pre-reduction at the first meiotic division.

Wenrich (1916) and Carothers (1931) have described both pre- and post-reduction in heteromorphic chromosomes. According to Wenrich (figure 65) chromosome "C" in *Phrynotettix* forms only a single chiasma. One of the homologues is shorter than the other, and the two short chromatids are always paired. In about half the cases the first meiotic division is reductional, and in half the cases it is equational. On any theory of chiasma formation it would be impossible to obtain both types of reduction with only a single chiasma and a constant association of short chromatids unless the spindle fiber can be attached more or less at random to either end of the homologous chromosomes. But the spindle fiber attachment point seems to be constant for individual chromosomes of both plants and animals. In Wenrich's figure 64, showing the behavior of the unequal chromosome pair B, the segregation is equational, but in order to have an equational division, it would be necessary to assume that non-sister threads are paired at the fiber constriction or that a crossover has occurred in all cases.

Similar heteromorphic chromosomes in *Trimerotropis* have been described by Carothers (1931). The unequal homologues may undergo either pre-reduction or post-reduction, although in the few figures showing chromatid association, only a single chiasma is present, and the short chromatids are always paired. If the first meiotic division can be either pre-reductional or post-reductional, it is necessary to assume that some crossing over occurs if sister chromatids are always paired at the point of spindle fiber attachment. The behavior of unequal homologues should receive further study in connection with the implications involved concerning crossing over and the nature of chiasma formation.

SUMMARY

If chiasmata are formed by the alternate pairing of sister and non-sister chromatids, each chromatid may lie in the same quadrant at all loci. An asymmetrical relation of the chromatids can exist only if sister chromatids are twisted before or after synapsis or if a crossover has occurred.

If chiasmata are the result of previous crossovers, the chromatids should not lie in the same quadrants at all loci, and symmetrical arrangements of the chromatids should seldom be found.

In *Pæonia suffruticosa* the homologous chromosomes may pair at meiosis without chiasma formation. When chiasmata are found, the chromatids are often symmetrical in the bivalent chromosomes. The symmetrical relations of the chromatids in bivalents with two chiasmata are very difficult to explain on the partial chiasmatypy hypothesis. These figures are essentially the same as the types found in Orthopteran species.

Most of the asymmetrical configurations found in the *Pæonia* chromosomes could be attributed to half twists in paired sister chromatids. Some chiasmata are found, however, where the chromatids forming the cross are adjacent on both sides of the chiasma. Such a relation of the chromatids supports the partial chiasmatypy hypothesis of chiasma formation.

Occasionally one or more homologous chromosomes are not paired at the first meiotic metaphase, but pass to the poles as univalents. The segregation of two homologues to the same pole would result in genetic "non-disjunction."

In some cases homologous chromosomes are paired without chiasma formation.

Pre- and post-reduction divisions are discussed in relation to the nature of chiasma formation.

Arnold Arboretum
Harvard University

LITERATURE CITED

Bělař, K. (1928). Die cytologischen Grundlagen der Vererbung. pp. 412. Borntræger, Berlin.

Belling, J. (1931). Chiasmas in flowering plants. (Univ. Calif. Pub. Bot. 16: 311–338.)

Bridges, C. B. & E. G. Anderson (1925). Crossing over in the X chromosomes of triploid females of Drosophila melanogaster. (Genetics, **10**: 418–441.)

Carothers, E. E. (1926). The maturation divisions in relation to the segregation of homologous chromosomes. (Quart. Rev. Biol. **1**: 419–435.)

————. (1931). The maturation divisions and segregation of heteromorphic homologous chromosomes in Acrididæ (Orthoptera). (Biol. Bull. **61**: 324–349.)

Creighton, H. B. & B. McClintock (1931). A correlation of cytological and genetical crossing-over in Zea Mays. (Proc. Nat. Acad. Sci. **17**: 492–497.)

Darlington, C. D. (1930). A cytological demonstration of crossing over. (Proc. Roy. Soc. B. **107**: 50–59.)

————(1931). Meiosis. (Biol. Rev. **6**: 221–264.)

Darlington, C. D. & S. O. S. Dark (1932). The origin and behavior of chiasmata. II. Stenobothrus parallelus. (Cytologia, **3**: 169–185.)